

SPECIFICATION FOR REISSUE OF U.S. PATENT NO. 5,695,159

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ETHERLIPID-CONTAINING MULTIPLE LIPID LIPOSOMES

This application is a CIP of Ser. No. filed Feb. 16, 1996 now U.S. Pat. No. 5,762,958.

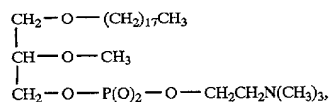
Etherlipids are synthetic analogues of platelet activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), an effector generally believed to be involved in a variety of physiological processes, such as inflammation, the immune response, allergic reactions and reproduction. Etherlipids have been shown to be effective antitumor agents in animals, and are believed to be selectively cytotoxic to a broad variety of cancer cells (see, for example, Dietzfelbinger et al. (1993); Zeisig et al. (1993); Powis et al. (1990); Berdel (1991); Bhatia and Hadju (1991); Reed et al. (1991); Workman (1991); Workman et al. (1991); Bazill and Dexter (1990); Berdel (1990); Counsell et al. (1990); Tritton and Hickman (1990); Muschiol et al. (1990); Layton et al. (1980); Runge et al. (1980); Great Britain Patent No. 1,583,661; U.S. Pat. No. 3,752,886). Etherlipids have also been shown to be antitumorigenic and anti-invasive, and to be capable of cell differentiation induction.

Mechanisms of etherlipid cytotoxicity, while not definitively established, appear to involve action at, and possible disruption of, the cell membrane. The selective cytotoxicity of etherlipids may involve intracellular accumulation and differential activity of alkyl cleavage enzymes. Etherlipids may also be selective inhibitors of phosphatidylinositol phospholipase C and protein kinase C activities, as well as of phosphatidylcholine biosynthesis. Hence, etherlipids are potentially quite useful as therapeutic agents. However, their administration can also lead to hemolysis, hepatic dysfunction and gastrointestinal disorders. Applicants have found that certain liposomal formulations of etherlipids can buffer these toxicities without inhibiting anticancer efficacy, and thereby can provide a more therapeutically useful basis for etherlipid administration.

SUMMARY OF THE INVENTION

This invention provides a liposome comprising a bilayer having a lipid component which comprises: (a) a phosphatidylcholine; (b) a sterol; (c) a headgroup derivatized lipid and, (d) an etherlipid. The headgroup-derivatized lipid, comprising a phosphatidylethanolamine linked to a moiety selected from the group consisting of dicarboxylic acids, polyethylene glycols, gangliosides and polyalkylethers, comprises from about 5 mole percent to about 20 mole percent of the bilayer's lipid component; the etherlipid comprises from about 10 mole percent to about 30 mole percent of the lipid component.

Preferably, the phosphatidylcholine is dioleoyl phosphatidylcholine ("DOPC"), the sterol is cholesterol ("chol"), the headgroup-derivatized lipid is dioleoyl phosphatidylethanolamine-glutaric acid ("DOPE-GA") and the etherlipid is



also known as "EL-18," "ET-18-OCH₃," or "edelfosine". Most preferably, the liposome is a unilamellar liposome having a diameter of from greater than about 50 nm to less than about 200 nm, and the liposome's bilayer has a lipid component comprising about 20 mole percent of the

etherlipid, about 10 mole percent of the headgroup-derivatized lipid, about 30 mole percent cholesterol and about 40 mole percent dioleoyl phosphatidylcholine.

Also provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and such liposomes. Further provided is a method of treating a mammal afflicted with a cancer, including, but not limited to: a lung, brain, colon, ovarian or breast cancers, the method comprising administering the pharmaceutical compositions of this invention to the mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

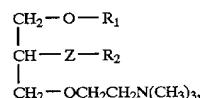
FIG. 1. Time Course of Carboxyfluorescein Leakage from Liposomal Edelfosine Formulations Incubated at 48 deg. Celsius in PBS. ELL 28 (uppermost curve, "ELL" indicating "etherlipid liposome"): Distearoyl phosphatidylcholine ("DSPC"); cholesterol ("CHOL"); dioleoyl phosphatidylethanolamine-glutaric acid ("DOPE-GA"); edelfosine "EL," standing for "etherlipid" (the respective molar ratio of these lipid components being 4:3:1:2); ELL 30 (second from top curve): EPC:CHOL:DOPE-GA:EL (4:3:1:2); ELL 25 (middle curve): DOPE:CHOL:DOPE-GA:EL (3:3:1:3); ELL 12 (second from bottom curve): DOPC:CHOL:DOPE-GA:EL (4:3:1:2); and, ELL 20 (bottom curve): DOPE:CHOL:DOPE-GA:EL (4:3:1:2). Y-axis: % CF Leakage; x-axis: time (seconds).

FIG. 2. Comparison of Hemolytic Activity and CF Leakage in Etherlipid Liposomes. From top-to-bottom: ELL 20—ELL 12—ELL 25—ELL 30—ELL 28 ($y=34231x^{-2.1614}$; $R^2=0.96$). Y-axis: H₁₀; x-axis: % CF leakage upon incubation in PBS.

FIG. 3. Stability of Etherlipid Liposomal Formulations on Incubation in 0.5% Serum at 37 Degrees Celsius. Y-axis: time (minutes); x-axis (from left-to-right): ELL 28, ELL 40, ELL 30; ELL 25; ELL 12; ELL 20. Inset: Y-axis: time (minutes); x-axis: ELL 28, ELL 40, ELL 30.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a liposome comprising a bilayer having a lipid component which comprises: (a) a phosphatidylcholine; (b) a sterol; (c) a headgroup derivatized lipid containing a phosphatidylethanolamine and a moiety selected from the group consisting of dicarboxylic acids, gangliosides, polyethylene glycols and polyalkylethers, which headgroup-derivatized lipid comprises from about 5 mole percent to about 20 mole percent of the bilayer's lipid component; and, (d) an etherlipid having the following formula:



the etherlipid comprising from greater than about 10 mole percent, to less than about 30 mole percent, of the bilayer's lipid component.

"Liposomes" are self-assembling structures comprising one or more lipid bilayers, each of which surrounds an aqueous compartment and comprises two opposing monolayers of amphipathic lipid molecules. Amphipathic lipids comprise a polar (hydrophilic) headgroup region covalently linked to one or two non-polar (hydrophobic) acyl chains. Energetically unfavorable contacts between the hydrophobic

acyl chains and the aqueous medium are generally believed to induce lipid molecules to rearrange such that the polar headgroups are oriented towards the aqueous medium while the acyl chains reorient towards the interior of the bilayer. An energetically stable structure is formed in which the acyl chains are effectively shielded from coming into contact with the aqueous medium.

Liposomes can have a single lipid bilayer (unilamellar liposomes, "ULVs"), or multiple lipid bilayers (multilamellar liposomes, "MLVs"), and can be made by a variety of methods (for a review, see, for example, Deamer and Uster (1983)). These methods include without limitation: Bangham's methods for making multilamellar liposomes (MLVS); Lenk's, Fountain's and Cullis' methods for making MLVs with substantially equal interlamellar solute distribution (see, for example, U.S. Pat. Nos. 4,522,803, 4,588,578, 5,030,453, 5,169,637 and 4,975,282); and Papahadjopoulos et al.'s reverse-phase evaporation method (U.S. Pat. No. 4,235,871) for preparing oligolamellar liposomes. ULVs can be produced from MLVs by such methods as sonication (see Papahadjopoulos et al. (1968)) or extrusion (U.S. Pat. No. 5,008,050 and U.S. Pat. No. 5,059,421). The etherlipid liposome of this invention can be produced by the methods of any of these disclosures, the contents of which are incorporated herein by reference.

Various methodologies, such as sonication, homogenization, French Press application and milling can be used to prepare liposomes of a smaller size from larger liposomes. Extrusion (see U.S. Pat. No. 5,008,050) can be used to size reduce liposomes, that is to produce liposomes having a predetermined mean size by forcing the liposomes, under pressure, through filter pores of a defined, selected size. Tangential flow filtration (see WO89/008846), can also be used to regularize the size of liposomes, that is, to produce liposomes having a population of liposomes having less size heterogeneity, and a more homogeneous, defined size distribution. The contents of these documents are incorporated herein by reference. Liposome sizes can also be determined by a number of techniques, such as quasi-electric light scattering, and with equipment, e.g., Nicomp® particle sizers, well within the possession of ordinarily skilled artisans.

The liposomes of this invention can be unilamellar or multilamellar. Preferably the liposomes are unilamellar and have diameters of less than about 200 nm, more preferably, from greater than about 50 nm to less than about 200 nm; such liposomes are preferably produced by a method comprising the steps of: dissolving lipids in a suitable organic solvent so as to establish a lipidic solution; removing the organic solvent from the resulting lipidic solution; adding an aqueous solution so as to form liposomes; and, then extruding the resulting liposomes through a suitable filter.

Liposomes of the 50–200 nm size are preferred because they generally believed to circulate longer in mammals than do larger liposomes, which are more quickly recognized by the mammals' reticuloendothelial systems ("RES"), and hence, more quickly cleared from the circulation. Longer circulation can enhance therapeutic efficacy by allowing more liposomes to reach their intended site of actions, e.g., tumors or inflammations. Small unilamellar liposomes, i.e., those generally less than 50 nm in diameter, carry amounts of bioactive agents which may be, in some cases, too low to be of sufficient therapeutic benefit.

R_1 of the etherlipid, the chain attached at the carbon #1 position of its glycerol backbone by way of an oxygen, has the formula $Y_1[Y_2]Y_3$. Y_2 is CH_3 or CO_2H , but preferably is CH_3 . Y_1 is $-(CH_2)_{n1}(CH=CH)_{n2}(CH)_{n3}(CH=CH)_{n4}(CH_2)_{n5}$

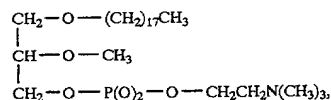
$(CH=CH)_{n6}(CH_2)_{n7}(CH=CH)_{n8}(CH_2)_{n9}$; the sum of $n1+2n2+n3+2n4+n5+2n6+n7+2n8+n9$ is an integer of from 3 to 23; that is, the acyl chain is from 4–24 carbon atoms in length. $n1$ is equal to zero or is an integer of from 1 to 23; $n3$ is equal to zero or is an integer of from 1 to 20; $n5$ is equal to zero or is an integer of from 1 to 17; $n7$ is equal to zero or is an integer of from 1 to 14; $n9$ is equal to zero or is an integer of from 1 to 11; and each of $n2$, $n4$, $n6$ and $n8$ is independently equal to zero or 1.

The hydrocarbon chain is preferably saturated, that is, it preferably has no double bonds between adjacent carbon atoms, each of $n2$, $n4$, $n6$ and $n8$ then being equal to zero. Accordingly, Y_1 is preferably $(CH_2)_{n1}$. More preferably, R_1 is $(CH_2)_{n1}CH_3$, and most preferably, is $(CH_2)_{17}CH_3$. Alternatively, the chain can have one or more double bonds, that is, it can be unsaturated, and one or more of $n2$, $n4$, $n6$ and $n8$ can be equal to 1. For example, when the unsaturated hydrocarbon has one double bond, $n2$ is equal to 1, $n4$, $n6$ and $n8$ are each equal to zero and Y_1 is $(CH_2)_{n1}CH=CH(CH_2)_{n3}$. $n1$ is then equal to zero or is an integer of from 1 to 21, and $n3$ is also zero or is an integer of from 1 to 20, at least one of $n1$ or $n3$ not being equal to zero.

Z is oxygen, sulfur, NH, or $-NHC(O)-$, Z then being connected to the methyl group by way of either the nitrogen or carbonyl carbon. Z can also be $-OC(O)-$, it then being connected to the methyl group by way of either the oxygen or carbonyl carbon atom. Preferably, Z is O; accordingly, this invention's glycerol-based etherlipids preferably have a methoxy group at the sn-2 position of their glycerol backbone.

R_2 is an alkyl group, or a halogen-substituted alkyl group, having the formula $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$, wherein each of X_1 , X_2 , X_3 , X_4 , and X_5 is independently hydrogen or a halogen, but is preferably hydrogen. $n10$ is equal to zero, 1 or 2; $n11$ is equal to zero, 1, or 2; and $n12$ is equal to zero or an integer of from 1 to 23, but is most preferably, zero, R_2 then being $CX_3X_4X_5$. X_3 , X_4 , and X_5 are most preferably H, R_2 then being CH_3 . Accordingly, the etherlipid preferably has a methyl group attached to its carbon #2. However, R_2 can then also be CH_2F , CHF_2 or CF_3 . When $n12$ is not zero, the sum of $n10+n11$ is equal to 2, $n12$ is preferably equal to 1, and R_2 is preferably CH_2CH_3 , CH_2CF_3 or CF_2CF_3 .

Most preferably, the etherlipid is one in which Y_2 is CH_3 , R_1 is $(CH_2)_{n1}CH_3$, R_2 is CH_3 and Z is O. The preferred etherlipid is therefore:



that is, 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine ("ET-18-OCH₃" or "edelfosine").

Preferably, the phosphatidylcholine ("PC") is partially or wholly unsaturated, that is, it has two acyl chains, at least one of which has at least one double bond between adjacent carbon atoms. More preferably, presently, the PC is dioleoyl phosphatidylcholine ("DOPC"). The liposome's lipid bilayer also contains a sterol, which generally affects the fluidity of lipid bilayers (see, for example, Lewis and McElhaney (1992) and Darnell et al. (1986)). Accordingly, sterol interactions with surrounding hydrocarbon chains generally inhibit emigration of these chains from the bilayer. The sterol of the liposomes of this invention is preferably, but not necessarily, cholesterol, and can also be a variety of other sterolic compounds.

A "headgroup-derivatized" lipid is a lipid which, when present in a liposomal lipid bilayer with an etherlipid, can buffer the toxicity of the etherlipid. That is, the derivatized lipid can decrease the etherlipid's toxicity, such that it is generally less toxic than the free form of the etherlipid. Headgroup-derivatized lipids generally are amphipathic lipids comprising hydrophobic acyl chains, and a phosphorylethanolamine group to which a suitable chemical moiety has been attached. Acyl chains are those which can adopt compatible packing configurations with the hydrophobic portions of other lipids present in the bilayer, and which can interact with an etherlipid such that release of the etherlipid from the bilayer is inhibited and etherlipid toxicity is buffered; these are saturated or unsaturated, straight-chained or branched, and typically contain from 4 to 24 carbon atoms in a straight chain. Preferred acyl chains are palmitate or oleate chains; hence preferred headgroup-modified lipids are dipalmitoyl phosphatidylethanolamine ("DPPE"), palmitoyl-oleoyl phosphatidylethanolamine ("POPE") or dioleoyl phosphatidylethanolamine ("DOPE"); most preferably, presently, the lipid is DOPE.

Chemical moieties suitable for attachment to such lipids are those, such as dicarboxylic acids, gangliosides, polyethylene glycols, polyalkyl ethers and the like, which can be attached to the amino group of a phosphorylethanolamine, and which give rise to lipids having toxicity buffering, circulation-enhancing properties. Means of identifying suitable chemical moieties, for example by subjecting derivatized lipids to in vitro and in vivo toxicity testing, are well known to, and readily practiced by, ordinarily skilled artisans given the teachings of this invention. Means of attaching chemical moieties to phosphorylethanolamine groups are also well known to, and readily practiced by, ordinarily skilled artisans.

Toxicity buffering capacities of headgroup-derivatized lipids can be determined by a number of *in vitro* and *in vivo* testing methods well known to, and readily practiced by, ordinarily skilled artisans, given the teachings of this invention. For example, etherlipid-induced red blood cell (RBC) hemolysis can be examined *in vitro* by combining an etherlipid with an RBC suspension, incubating the combination, and then quantitating the percentage of RBC lysis.

Toxicity-buffering can also be assessed by determining the etherlipid's therapeutic window "TW," which is a numerical value derived from the relationship between the compound's induction of hemolysis and its ability to inhibit the growth of tumor cells. TW values are determined in accordance with the formula HI_5/GI_{50} (wherein " HI_5 " equals the concentration of compound inducing the hemolysis of 5% of the red blood cells in a culture, and wherein " GI_{50} " equals the dose of compound inducing fifty percent growth inhibition in a population of cells exposed to the agent). The higher an agent's HI_5 value, the less hemolytic is the agent—higher HI_5 's mean that greater concentrations of compound are required to be present in order for the compound to induce 5% hemolysis. Hence, the higher its HI_5 , the more therapeutically beneficial is a compound, because more of it can be given before inducing the same amount of hemolysis as an agent with a lower HI_5 . By contrast, lower GI_5 's indicate better therapeutic agents—a lower GI_{50} value indicates that a lesser concentration of an agent is required for 50% growth inhibition. Accordingly, the higher is its HI_5 value and the lower is its GI_{50} value, the better are a compound's agent's therapeutic properties.

Generally, when a bioactive agent's TW is less than 1, it cannot be used effectively as a therapeutic agent. That is, the agent's HI_5 value is sufficiently low, and its GI_{50} value

sufficiently high, that it is generally not possible to administer enough of the agent to achieve a sufficient level of tumor growth inhibition without also attaining an unacceptable level of hemolysis. Etherlipid liposomes having bilayers that also comprise headgroup-derivatized lipids have TS's of greater than 1. Preferably, the TW of an etherlipid in a liposomal bilayer also comprising a headgroup-derivatized lipid is greater than about 1.5, more preferably, greater than about 2, and still more preferably, greater than about 3.

Headgroup-derivatized lipids can also be circulation-enhancing lipids, that is, the modifications directed to lipid toxicity buffering can also afford circulation enhancement. Accordingly, headgroup-derivatized lipids can inhibit clearance of liposomes from the circulatory systems of animals to which they have been administered. Liposomes are generally believed to be cleared from an animal's body by way of its reticuloendothelial system (RES). Avoiding RES clearance means that the frequency of liposome administration can be reduced, and that less of a liposome-associated bioactive agent need be administered to achieve desired serum levels of the agent. Enhanced circulation times can also allow targeting of liposomes to non-RES containing tissues.

Liposome outer surfaces are believed to become coated with serum proteins, such as opsonins, in animals' circulatory systems. Without intending in any way to be limited by theory, it is believed that liposome clearance can be inhibited by modifying the outer surface of liposomes such that binding of serum proteins thereto is generally inhibited. Effective surface modification, that is, alterations to the outer surfaces of liposomes which result in inhibition of opsonization and RES uptake, is believed to be accomplished by incorporating into liposomal bilayers lipids whose polar headgroups have been derivatized by attachment thereto of a chemical moiety which can inhibit the binding of serum proteins to liposomes such that the pharmacokinetic behavior of the liposomes in the circulatory systems of animals is altered (see, e.g., Blume et al. (1993); Gabizon et al. (1993); Park et al. (1992); Woodlee et al. U.S. Pat. No. 5,013,556; and, U.S. Pat. No. 4,837,028).

Presently, dicarboxylic acids, such as glutaric, sebacic, succinic and tartaric acids, are preferred components of headgroup-derivatized lipids. Most preferably, the dicarboxylic acid is glutaric acid ("GA"). Accordingly, preferred headgroup-derivatized lipids include phosphatidylethanolamine-dicarboxylic acids such as dipalmitoyl phosphatidylethanolamine-glutaric acid ("DPPE-GA"), palmitoyloleoyl phosphatidylethanolamine-glutaric acid ("POPE-GA") and dioleoyl phosphatidylethanolamine-glutaric acid ("DOPE-GA"). Most preferably, presently, the derivatized lipid is DOPE-GA.

The liposomes of this invention can comprise one or more additional lipids, that is, lipids in addition to the phosphatidylcholine, sterol, headgroup-derivatized lipid and etherlipid already present in the liposomes' bilayers. Additional lipids are selected for their ability to adapt compatible packing conformations with the other lipid components of the bilayer such that the lipid constituents are tightly packed, and release of the lipids from the bilayer is inhibited. Lipid-based factors contributing to compatible packing conformations are well known to ordinarily skilled artisans and include, without limitation, acyl chain length and degree of unsaturation, as well as the headgroup size and charge. Accordingly, suitable additional lipids, including various phosphatidylethanolamines ("PE's") such as egg phosphatidylethanolamine ("EPE") or dioleoyl phosphatidylethanol-

The liposome of this invention can be dehydrated, stored and then reconstituted such that a substantial portion of its internal contents are retained. Liposomal dehydration generally requires use of a hydrophilic drying protectant such as a disaccharide sugar at both the inside and outside surfaces of the liposome bilayers (see U.S. Pat. No. 4,880,635). This hydrophilic compound is generally believed to prevent the rearrangement of the lipids in the liposome, so that the size and contents are maintained during the drying procedure and through subsequent rehydration. Appropriate qualities for

Preferably, the liposome administered is a unilamellar liposome having an average diameter of from about 50 nm to about 200 nm. The anti-cancer treatment method can

include administration of one or more bioactive agents in addition to the liposomal etherlipid, these additional agents preferably, but not necessarily, being included in the same liposome as the etherlipid. The additional bioactive agents, which can be entrapped in liposomes' internal compartments or sequestered in their lipid bilayers, are preferably, but not necessarily, anticancer agents or cellular growth promoting factors.

The liposomes are also effective as anti-inflammatory agents.

This invention will be better understood from the following examples. However, those of ordinary skill in the art will readily understand that these examples are merely illustrative of the invention as defined in the claims which follow thereafter.

EXAMPLES

Example 1

Preparation

Liposomes were prepared with edelfosine (ET-18-O-CH₃, 5 mg/ml), various other lipids obtained from Avanti Polar Lipids, Birmingham, Ala., and cholesterol (Sigma Chemical Co.). Briefly, the lipids were dissolved in an organic solvent, such as chloroform, at various mole ratios. The organic solvent was then removed, and the dried lipids were rehydrated, e.g., with Dulbecco's phosphate-buffered saline (D-PBS) (Gibco BRL Life Technologies, Grand Island, N.Y.). The resulting liposomes were extruded through 0.1 micron Nuclepore® filters (see, for example, Mayer et al., 1985). Liposome sizes were then determined by light scattering, using a Nicomp® Model 370 Submicron Particle Sizer.

Example 2

Red Blood Cell ("RBC") Hemolysis Assay

A 4% suspension of red blood cells (RBCs), 0.5 ml, was washed three times in PBS and then incubated with free (non-liposomal) etherlipid or liposomal etherlipid, prepared as described above. These samples were vortexed on a 37 deg. C. agitator for 20 hours, and were then centrifuged for 10 minutes at 3000 rpm. 0.2 ml of the resulting supernatant was diluted to 1 ml with water, and the percentage hemolysis in the sample was quantitated by spectrophotometric examination at 550 nm.

Results from these studies are presented in Table 1 (see below), wherein the concentration (μ M) of edelfosine required to cause 10% RBC hemolysis ("HI₁₀") in each formulation is set forth. The table's first column is a shorthand designation of the particular formulation, "ELL" standing for "etherlipid liposome." The second column indicates the components of the formulation tested, including dioleoyl phosphatidylethanolamine ("DOPE"), cholesterol ("CHOL"), dioleoyl-phosphatidylethanolamine-glutaric acid ("DOPE-GA"), dioleoyl phosphatidylcholine ("DOPC"), palmitoyl dioleoyl phosphatidylcholine ("POPC"), distearoyl phosphatidylcholine ("DSPC"), egg phosphatidylcholine ("EPC") and edelfosine ("EL," for etherlipid). The respective molar ratios of the various lipid components are also set forth. The last row of the table gives the HI₁₀ value for edelfosine alone, i.e., not incorporated in a liposome.

TABLE 1

Formulation	Composition	HI ₁₀
ELL 20	DOPE:CHOL:DOPE-GA:EL 4 3 1 2	1726 \pm 160
ELL 12	DOPC:CHOL:DOPE-GA:EL 4 3 1 2	670 \pm 60
ELL 40	POPC:CHOL:DOPE-GA:EL 4 3 1 2	65 \pm 6
ELL 28	DSPC:CHOL:DOPE-GA:EL 4 3 1 2	32 \pm 3
ELL 25	DOPE:CHOL:DOPE-GA:EL 3 3 1 3	537 \pm 50
ELL 30	EPC:CHOL:DOPE-GA:EL 4 3 1 2	314 \pm 30
Edelfosine	—	5 \pm 1

Example 3

Fluorescence Spectroscopy

Liposomes were prepared as described above, and in the presence of an aqueous solution of 0.1 M 6-carboxyfluorescein ("CF"); free CF was then removed by gel filtration. CF efflux from liposomes over time was monitored by measuring, at 520 nm (excitation at 490 nm), increases in CF fluorescence in the aqueous phase external to the liposomes, upon their incubation in PBS at 48 deg. C. Fluorescence values, presented in FIG. 1 herein, are expressed as a percentage increase in CF fluorescence relative to the total CF fluorescence found after disrupting liposomes with Triton X-100.

FIG. 2 herein compares hemolytic activity and CF leakage in various liposomal formulations described in Table 1, upon incubation of the liposomes in PBS at 48 deg. C. for 25 minutes. FIG. 3 compares the time required for 50% CF leakage in various liposomal formulations, upon their incubation in 0.5% serum at 37 deg. C.

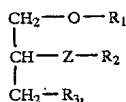
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What is claimed is:

1. A liposome having a lipid bilayer which comprises: (a) a phosphatidylcholine; (b) a sterol; (c) a headgroup-derivatized lipid comprising a phosphatidylethanolamine linked at the ethanolamine group to a dicarboxylic acid; and, (d) an etherlipid having the formula:



wherein R_1 is Y_1Y_2 , Y_2 is CH_3 or CO_2H , Y_1 is $(\text{CH}_2)_{n1}$ ($\text{CH}=\text{CH}$) $_{n2}$ (CH_2) $_{n3}$ ($\text{CH}=\text{CH}$) $_{n4}$ (CH_2) $_{n5}$ ($\text{CH}=\text{CH}$) $_{n6}$ (CH_2) $_{n7}$ ($\text{CH}=\text{CH}$) $_{n8}$ (CH_2) $_{n9}$, the sum of $n1+2n2+n3+2n4+n5+2n6+n7+2n8+n9$ is an integer of from 3 to 23, $n1$ is zero or an integer of from 1 to 23, $n3$ is zero or an integer of from 1 to 20, $n5$ is zero or an integer of from 1 to 17, $n7$ is zero or an integer of from 1 to 14, $n9$ is zero or an integer of from 1 to 11, and each of $n2$, $n4$, $n6$ and $n8$ is independently zero or 1;

wherein Z is oxygen or sulfur and R_2 is CH_3 ;

wherein R_3 is $-\text{O}-\text{P}(\text{O})_2-\text{O}-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$;

and wherein the phosphatidylethanolamine-dicarboxylic acid comprises from about 5 mole percent to about 20 mole percent of the lipid bilayer and the etherlipid comprises from greater than about 10 mole percent to less than about 30 mole percent of the lipid bilayer.

2. The liposome of claim 1 which is a unilamellar liposome having a diameter of from greater than about 50 nm to less than about 200 nm.

3. The liposome of claim 1, wherein the phosphatidylcholine is an unsaturated or partially unsaturated phosphatidylcholine.

4. The liposome of claim 3, wherein the phosphatidylcholine is dioleoyl phosphatidylcholine.

5. The liposome of claim 1, wherein the sterol is cholesterol.

6. The liposome of claim 1, wherein the headgroup derivatized lipid comprises a phosphatidylethanolamine selected from the group consisting of dipalmitoyl phosphatidylethanolamine, palmitoyloleoyl phosphatidylethanolamine and dioleoyl phosphatidylethanolamine.

7. The liposome of claim 6, wherein the headgroup derivatized lipid comprises dioleoyl phosphatidylethanolamine.

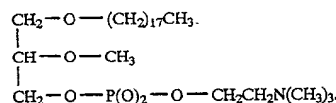
8. The liposome of claim 1, wherein the headgroup-derivatized lipid comprises a dicarboxylic acid selected from the group consisting of glutaric acid, sebacic acid, succinic acid and tartaric acid.

9. The liposome of claim 8, wherein the dicarboxylic acid is glutaric acid.

10. The liposome of claim 1, wherein the headgroup-derivatized lipid comprises dioleoyl phosphatidylethanolamine and glutaric acid.

11. The liposome of claim 1, wherein R_1 is $(\text{CH}_2)_{n1}\text{CH}_3$ and Z is O.

12. The liposome of claim 11, wherein the etherlipid is:



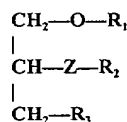
13. The liposome of claim 12, wherein the phosphatidylcholine is dioleoyl phosphatidylethanolamine, the sterol is cholesterol and the headgroup derivatized lipid comprises dioleoyl phosphatidylethanolamine and glutaric acid.

14. The liposome of claim 13, wherein the bilayer comprises about 20 mole percent of the etherlipid, about 10 mole percent of the headgroup-derivatized lipid, about 30 mole percent cholesterol and about 40 mole percent dioleoyl phosphatidylcholine.

15. The liposome of claim 1, comprising an additional bioactive agent.

16. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the liposome of claim 1.

17. A liposome having a lipid bilayer which comprises: (a) a phosphatidylcholine; (b) a sterol; (c) a headgroup-derivatized lipid comprising a phosphatidylethanolamine linked at the ethanolamine group to a dicarboxylic acid; and, (d) an etherlipid having the formula:



wherein R_1 is Y , Y_1 , Y_2 is CH_2 or CO_2H , Y_1 is $(CH_2)_{n1}(CH=CH)_{n2}(CH_2)_{n3}(CH=CH)_{n4}(CH_2)_{n5}(CH=CH)_{n6}(CH_2)_{n7}(CH=CH)_{n8}(CH_2)_{n9}$, the sum of $n1 + 2n2 + n3 + 2n4 + n5 + 2n6 + n7 + 2n8 + n9$ is an integer of from 3 to 23, $n1$ is zero or an integer of from 1 to 23, $n3$ is zero or an integer of from 1 to 20, $n5$ is zero or an integer of from 1 to 17, $n7$ is zero or an integer of from 1 to 14, $n9$ is zero or an integer of from 1 to 11, and each of $n2$, $n4$, $n6$ and $n8$ is independently zero or 1;

wherein Z is NH , $C(O)O$, or $HNC(O)$;
 R_2 is CH_2 ;
 R_3 is $-O-P(O)(-O-CH_2CH_2N(CH_3)_3)$; and

wherein the phosphatidylethanolamine-dicarboxylic acid comprises from about 5 mole percent to about 20 mole percent of the lipid bilayer and the etherlipid comprises from greater than about 10 mole percent to less than about 30 mole percent of the lipid bilayer.

18. The liposome of claim 17 which is a unilamellar liposome having a diameter of from greater than about 50 nm to less than about 200 nm.

19. The liposome of claim 17, wherein the phosphatidylcholine is an unsaturated or partially unsaturated phosphatidylcholine.

20. The liposome of claim 19, wherein the phosphatidylcholine is dioleoyl phosphatidylcholine.

21. The liposome of claim 17, wherein the sterol is cholesterol.

22. The liposome of claim 17, wherein the headgroup derivatized lipid comprises a phosphatidylethanolamine selected from the group consisting of dipalmitoyl phosphatidylethanolamine, palmitoyloleoyl phosphatidylethanolamine and dioleoyl phosphatidylethanolamine.

23. The liposome of claim 22, wherein the headgroup derivatized lipid comprises dioleoyl phosphatidylethanolamine.

24. The liposome of claim 17, wherein the headgroup-derivatized lipid comprises a dicarboxylic acid selected from the group consisting of glutaric acid, sebacic acid, succinic acid and tartaric acid.

25. The liposome of claim 24, wherein the dicarboxylic acid is glutaric acid.

26. The liposome of claim 17, wherein the headgroup-derivatized lipid comprises dioleoyl phosphatidylethanolamine and glutaric acid.

27. The liposome of claim 17, wherein R_1 is $(CH_2)_{n1}CH_3$ and Z is $C(O)O$.

28. The liposome of claim 17, wherein R_1 is $(CH_2)_{n1}CH_3$ and Z is NH .

29. The liposome of claim 17, wherein R_1 is $(CH_2)_{n1}CH_3$ and Z is $HNC(O)$.

30. The liposome of claim 17, comprising an additional bioactive agent.

31. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the liposome of claim 17.